DOCKET NO.: ISIS0003-101 (ISIS-5030) **PATENT**

AMENDMENTS TO THE CLAIMS:

Please cancel claims 38, 39, 59, 60, 72 and 73 without prejudice.

Please amend claims 1, 33, 55, 61-63, 65, 67 and 93 as follows:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1. **(currently amended)** A method for eliciting modification of a selected RNA target in a cell comprising:

(a) providing ~~an~~ a single-stranded RNA-like polynucleotide hybridizable with said RNA target;

(b) hybridizing the RNA-like polynucleotide and the RNA to form a polynucleotide-target duplex; and

(c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect modification of the duplex by the polypeptide, and modification of the RNA target thereby.

2. **(original)** The method of claim 1 wherein said modification of the RNA target occurs in the cell's nucleus.

3. **(original)** The method of claim 1 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.

4. **(original)** The method of claim 1 wherein the RNase III polypeptide is a human RNase III polypeptide.

5. **(original)** The method of claim 1 wherein modification of the selected RNA target is cleavage of the RNA target.

6. **(original)** The method of claim 1 wherein the polypeptide comprising an RNase III domain is present in enriched amounts.

7. **(original)** The method of claim 6 wherein the polypeptide comprising an RNase III

domain present in enriched amounts is overexpressed or exogenously added.

8. **(original)** The method of claim 1 wherein the polypeptide comprising an RNase III domain is a purified RNase III polypeptide.

9. **(original)** The method of claim 1 wherein the RNA-like polynucleotide has a modification at the 2' position of at least one sugar.

10. **(original)** The method of claim 1 wherein step (c) is performed within a cell.

11. **(original)** The method of claim 1 wherein step (b) is performed within a cell.

12. **(original)** The method of claim 1 wherein step (b) is performed outside a cell.

13. **(original)** The method of claim 1 wherein at least one furanosyl moiety of the RNA-like polynucleotide is a ribofuranosyl moiety.

14. **(original)** The method of claim 13 wherein a majority of the furanosyl moieties of the RNA-like polynucleotide are ribofuranosyl moieties.

15. **(original)** A method for promoting gene silencing in a cell comprising providing to the cell, in an amount effective to promote said gene silencing, a polypeptide comprising an RNase III domain.

16. **(original)** The method of claim 15 wherein said promotion of gene silencing occurs in the cell's nucleus.

17. **(original)** The method of claim 15 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.

18. **(original)** The method of claim 15 wherein the RNase III polypeptide is a human RNase III polypeptide.

19. **(original)** The method of claim 15 wherein the RNase III polypeptide is exogenously added.

20. **(original)** The method of claim 15 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.

21. **(original)** The method of claim 15 wherein said RNase III polypeptide is a purified RNase III polypeptide.

22. **(original)** The method of claim 15 wherein said RNase III polypeptide is expressed by an exogenously added vector encoding said RNase III polypeptide.

23. **(original)** The method of claim 15 wherein said cell is a mammalian cell.

24. **(original)** The method of claim 15 wherein said cell is a human cell.

25. **(original)** A method for promoting gene silencing in a cell comprising enriching the amount or activity of RNase III polypeptide in said cell to a level effective to promote said gene silencing.

26. **(original)** The method of claim 25 wherein said promotion of gene silencing occurs in the cell's nucleus.

27. **(original)** The method of claim 25 wherein said enriching is by exogenous addition of RNase III polypeptide.

28. **(original)** The method of claim 27 wherein said exogenously added RNase III polypeptide is a purified RNase III polypeptide.

29. **(original)** The method of claim 25 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.

30. **(original)** The method of claim 25 wherein said enriching is by addition of a vector encoding the RNase III polypeptide.

31. **(original)** The method of claim 25 wherein said cell is a mammalian cell.

32. **(original)** The method of claim 25 wherein said cell is a human cell.

33. **(currently amended)** A method for promoting gene silencing of a gene in a cell comprising:

(a) providing to said cell a single-stranded polynucleotide hybridizable with a target RNA encoded by a selected gene whose expression is to be silenced;

(b) hybridizing said polynucleotide and said target RNA to form a polynucleotide-target duplex; and

(c) contacting said duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect cleavage or modification of the target RNA strand of the polynucleotide-target RNA duplex by the polypeptide comprising an RNase III domain, and silencing of the gene thereby.

34. **(original)** The method of claim 33 wherein said promotion of gene silencing occurs in the cell's nucleus.

35. **(original)** The method of claim 33 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.

36. **(original)** The method of claim 33 wherein the RNase III polypeptide is a human RNase III polypeptide.

37. **(original)** The method of claim 36 wherein the human RNase III polypeptide comprises an amino acid sequence with at least 90% homology to SEQ ID NO: 2.

38. **(cancelled)**

39. **(cancelled)**

40. **(original)** The method of claim 33 wherein the polynucleotide is an antisense oligonucleotide.

41. **(original)** The method of claim 33 wherein the polynucleotide is an RNA-like polynucleotide.

42. **(original)** The method of claim 33 wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.

43. **(original)** The method of claim 42 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.

44. **(original)** The method of claim 33 wherein the polynucleotide has at least one modification of the base, sugar or internucleoside linkage.

45. **(original)** The method of claim 44 wherein the polynucleotide has a modification at the 2' position of at least one sugar.

46. **(original)** The method of claim 33 wherein the RNase III polypeptide is present in enriched amounts.

47. **(original)** The method of claim 46 wherein the RNase III polypeptide present in enriched amounts is overexpressed or exogenously added.

48. **(original)** The method of claim 46 wherein the RNase III polypeptide is a purified RNase III polypeptide.

49. **(original)** The method of claim 46 wherein said enriching is by addition of a vector encoding said RNase III polypeptide.

50. **(original)** The method of claim 46 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the polypeptide.

51. **(original)** The method of claim 33 wherein said cell is a mammalian cell.

52. **(original)** The method of claim 33 wherein said cell is a human cell.

53. **(original)** The method of claim 33 wherein said polynucleotide-target RNA duplex forms inside the cell.

54. **(original)** The method of claim 33 wherein said polynucleotide-target RNA duplex forms outside the cell.

55. **(currently amended)** A method for inhibiting the expression of a gene in a cell comprising providing to said cell an agent effective to elicit RNase III modification of double-stranded RNA in a the cell, wherein the agent, when a polynucleotide, is single-stranded.

56. **(original)** The method of claim 55 wherein said inhibition of gene expression occurs in the cell's nucleus.

57. **(original)** The method of claim 55 wherein said agent is a nucleic acid which is hybridizable with an RNA encoded by the gene whose expression is to be inhibited.

58. **(original)** The method of claim 55 wherein said RNase III modification is RNase III cleavage.

59. **(cancelled)**

60. **(cancelled)**

61. **(currently amended)** The method of claim 55 wherein the agent ~~polynucleotide~~ is an antisense oligonucleotide.

62. **(currently amended)** The method of claim 55 wherein the agent ~~polynucleotide~~ is an RNA-like polynucleotide.

63. **(currently amended)** The method of claim 55 wherein the agent is a polynucleotide and wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.

64. **(original)** The method of claim 63 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.

65. **(currently amended)** The method of claim 55 wherein the agent is a polynucleotide ~~has~~ having at least one modification of the base, sugar or internucleoside linkage.

66. **(original)** The method of claim 65 wherein the polynucleotide has a modification at the 2' position of at least one sugar.

67. **(currently amended)** A method for promoting inhibition of expression of a gene in a cell comprising:

(a) providing to said cell a single-stranded polynucleotide hybridizable with a target RNA encoded by the gene whose expression is to be inhibited;

(b) hybridizing the polynucleotide and the target RNA to ~~to~~ form a polynucleotide-target duplex; and

(c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions effective to effect cleavage or modification of the target RNA strand of the polynucleotide-target RNA duplex by the RNase III polypeptide, and inhibition of expression of the gene thereby.

68. **(original)** The method of claim 67 wherein said promotion of inhibition of gene expression occurs in the cell's nucleus.

69. **(original)** The method of claim 67 wherein the polypeptide comprising an RNase III domain is an RNase III polypeptide.

70. **(original)** The method of claim 69 wherein the RNase III polypeptide is a human RNase III polypeptide.

71. **(original)** The method of claim 70 wherein the human RNase III polypeptide comprises an amino acid sequence with at least 90% sequence identity to SEQ ID NO: 2.

72. **(cancelled)**

73. **(cancelled)**

74. **(original)** The method of claim 67 wherein the polynucleotide is an antisense oligonucleotide.

75. **(original)** The method of claim 67 wherein the polynucleotide is an RNA-like polynucleotide.

76. **(original)** The method of claim 67 wherein at least one sugar moiety of the polynucleotide is a ribofuranosyl sugar moiety.

77. **(original)** The method of claim 76 wherein at least 50% of the sugar moieties of the polynucleotide are ribofuranosyl sugar moieties.

78. **(original)** The method of claim 67 wherein the polynucleotide has at least one modification of the base, sugar or internucleoside linkage.

79. **(original)** The method of claim 78 wherein the polynucleotide has a modification at the 2' position of at least one sugar.

80. **(original)** The method of claim 67 wherein the polypeptide comprising an RNase III domain is present in enriched amounts.

81. **(original)** The method of claim 80 wherein the polypeptide comprising an RNase III

domain and present in enriched amounts is overexpressed or exogenously added.

82. **(original)** The method of claim 81 wherein the polypeptide comprising an RNase III domain and present in enriched amounts is a purified RNase III polypeptide.

83. **(original)** The method of claim 81 wherein said enriching is by addition of a vector encoding said polypeptide comprising an RNase III domain.

84. **(original)** The method of claim 67 wherein said cell is a human cell.

85. **(original)** The method of claim 67 wherein step (c) is performed within a cell.

86. **(original)** The method of claim 67 wherein step (b) is performed within a cell.

87. **(original)** The method of claim 67 wherein step (b) is performed outside a cell.

88. **(original)** A cell having enhanced RNase III activity over an activity exhibited by a second cell, said second cell not enriched with respect to the amount or activity of RNase III polypeptide.

89. **(original)** The cell of claim 88 wherein said enhanced RNase III activity is detectable in the cell's nucleus.

90. **(original)** The cell of claim 88 wherein said enhanced RNase III activity is due to overexpression of RNase III.

91. **(original)** The cell of claim 88 wherein the RNase III polypeptide is provided through upregulation of endogenous production of the RNase III polypeptide.

92. **(original)** The cell of claim 88 wherein said enhanced RNase III activity is due to exogenously added RNase III.

93. **(currently amended)** A method for eliciting modification of an RNA target in a cell comprising:

(a) providing ~~an~~ a single-stranded RNA-like polynucleotide hybridizable with said RNA target;

(b) hybridizing the RNA-like polynucleotide and the RNA to form a polynucleotide-target duplex; and

(c) contacting the duplex with a polypeptide comprising an RNase III domain, under conditions selected to effect modification of the duplex by the polypeptide, and modification of the RNA target thereby.

94. **(original)** A hybrid RNase III comprising at least one domain from a human RNase III and at least one domain from an RNase III of an organism other than human.

95. **(original)** The hybrid RNase III of claim 94 wherein the non-human RNase III domain is derived from an organism selected from the group consisting of E. coli, S. pombe, C. elegans and S. cerevisiae.